

FIG. 1

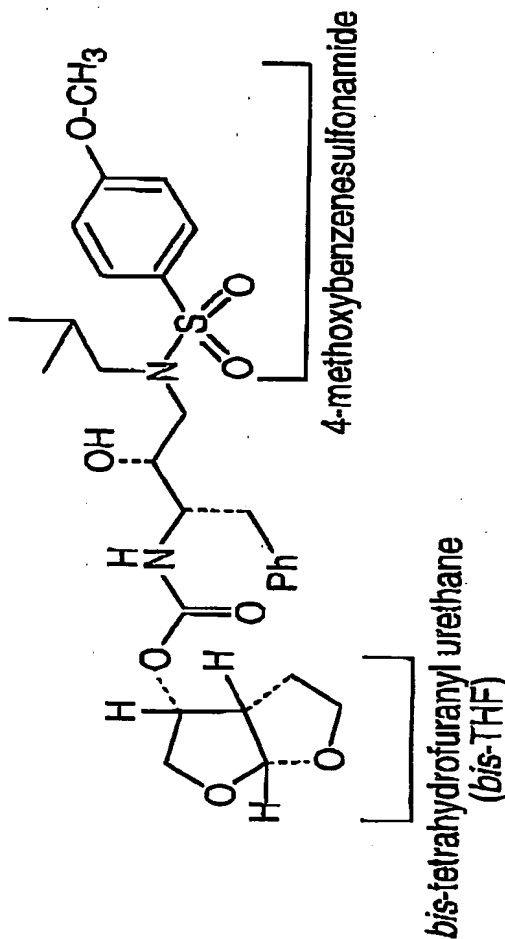
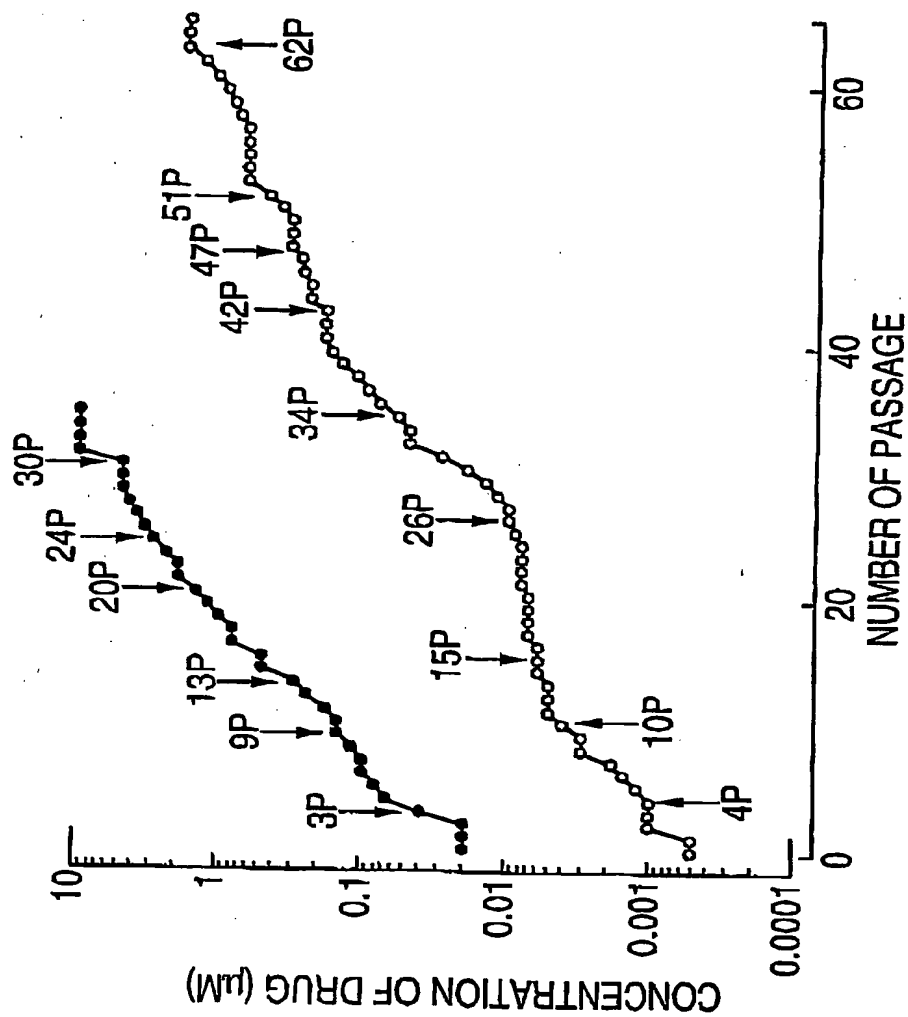


FIG. 2



10/500888

3/13

FIG. 3A

	10	20	30	40	50	60	70	80	90	99	FRACTION OF CLONES
pML-4-3	VTIKIGGOLK	EALDTGADD	TYLEENWLP	RKKPKHIGGI	GGFKVRQYD	QILLIEGSHK	AIGTVLGP	PWWIGRML	TOIGCTLNF		
p4-1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8/8
p10-1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6/16
p10-2	.....	.....	.....	.....	.....	V.....	.....	.....	.....	.....	2/16
p10-3	.....	.....	.....	.....	.....	.....	T.....	.....	.....	.....	2/16
p10-4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1/16
p10-5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1/16
p10-6	.....	.....	.....	.....	R.....	.....	.....	.....	.....	.....	1/16
p10-7	.....	.....	.....	.....	.....	.....	S.....	.....	.....	.....	1/16
p10-9	.....	.....	.....	.....	.....	.....	.....	I.....	.....	.....	1/16
p10-10	.....	.....	.....	.....	.....	.....	.....	V.....	.....	.....	1/16
p15-1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2/9
p15-2	.....	.....	S.....	.....	.....	.....	.....	I.....	.....	.....	2/9
p15-3	.....	.....	A.....	.....	.....	.....	.....	.....	.....	.....	1/9
p15-4	.....	.....	S.....	.....	R.....	.....	T.....	.....	.....	.....	1/9
p15-5	.....	.....	S.....	.....	.....	.....	.....	.....	.....	.....	1/9
p15-6	.....	.....	S.....	.....	.....	.....	.....	.....	.....	.....	1/9
p15-7	.....	.....	.....	.....	.....	.....	.....	I.....	.....	.....	1/9
p26-1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3/10
p26-2	.....	.....	S.....	.....	.....	.....	.....	I.....	.....	.....	2/10
p26-3	.....	F.....	S.....	.....	.....	.....	.....	I.....	.....	.....	1/10
p26-4	.....	.....	S.....	.....	.....	.....	T.....	I.....	.....	.....	1/10
p26-5	.....	.....	S.....	.....	.....	.....	.....	.....	.....	.....	1/10
p26-6	.....	.....	S.....	.....	.....	.....	V.....	.....	.....	.....	1/10
p26-7	.....	R.....	.....	I.....	I.....	.....	V.....	.....	.....	.....	1/10

10/500888

4/13

FIG. 3B

P34-1	F	I...V	V	4/10
P34-2	F	S	V	3/10
P34-3	F	S	V	1/10
P34-4	F	S	T	1/10
P34-5	F	S	T	1/10
P42-1	F	I...V	V	4/16
P42-2	F	I...V	T	2/16
P42-3	F	I...V	V...E...	1/16
P42-4	F	I...V	T	1/16
P42-5	F	I...V	V...I...	1/16
P42-6	F	I...V	V	1/16
P42-7	I	I...	V	1/16
P42-8	F	S	V	1/16
P42-9	P...Q.F	S	V...H.	1/16
P42-10	F	S	I...	1/16
P42-11	F	S...I...R		1/16
P42-12	F	S...R...I...		1/16
P47-1	F	I...V	V	3/9
P47-2	F	V...I...V	V	1/9
P47-3	F	S...I...	V	1/9
P47-4	F	S...I...R...	V	1/9
P47-5	F	S...I...		1/9
P47-6	F	S...I...T...	D	1/9
P47-7	F	S...I...	D	1/9

5/13

10/500888

FIG. 3C

P51-1	.....F.....S.....I.....V.....	.....V.....	3/9
P51-2	.....F.....S.....I.....V.....	.....V.S.....	1/9
P51-5	.....F.....S.....I.....	.....V.....	1/9
P51-6	.....F.....S.....I.....	.....V.....	1/9
P51-7	.....F.....S.....I.....	.....V.....S.....	1/9
P51-8	.....F.....S.....I.....	.....L.....D.....	1/9
P51-9	.....F.....S.....I.....	.....D.....L.....	1/9
P62-1	.....F.....S.....I.....	.....D.....	2/11
P62-2	.....F.....S.....I.....	.....V.....D.....	1/11
P62-3	.....F.....S.....I.....E.....	.....V.....	1/11
P62-4	.....F.....S.....I.....	.....V.....	1/11
P62-5	.....F.....S.....I.....	.....V.....	1/11
P62-6	.....F.....S.....I.....	.....V.....D.....	1/11
P62-7	.....F.....S.....I.....V.....	.....V.....D.....	1/11
P62-8	.....P.....F.....S.....I.....V.....	.....V.....	1/11
P62-9	.....P.....F.....S.....I.....V.....	.....V.....	1/11
P62-10	.....F.....S.....I.....V.....	.....V.....	1/11

SEQUENCE ANALYSIS OF THE PROTEASE-ENCODING REGION OF HIV-1 PASSAGED IN THE PRESENCE OF UIC-94003. THE AMINO ACID SEQUENCES OF PROTEASE DEDUCED FROM NUCLEOTIDE SEQUENCES OF THE PROTEASE-ENCODING REGION OF HIV-1 CLONES DETERMINED AT NINE DIFFERENT PASSAGES ARE ILLUSTRATED. THE FRACTION OF CLONES EXAMINED IS INDICATED ON THE RIGHT. THE AMINO ACID SEQUENCE OF PROTEASE OF A WILD-TYPE pM4-3 CLONE IS SHOWN AS A REFERENCE. IDENTITY WITH THIS SEQUENCE AT INDIVIDUAL AMINO ACID POSITIONS IS INDICATED (DOTS).

FIG. 4

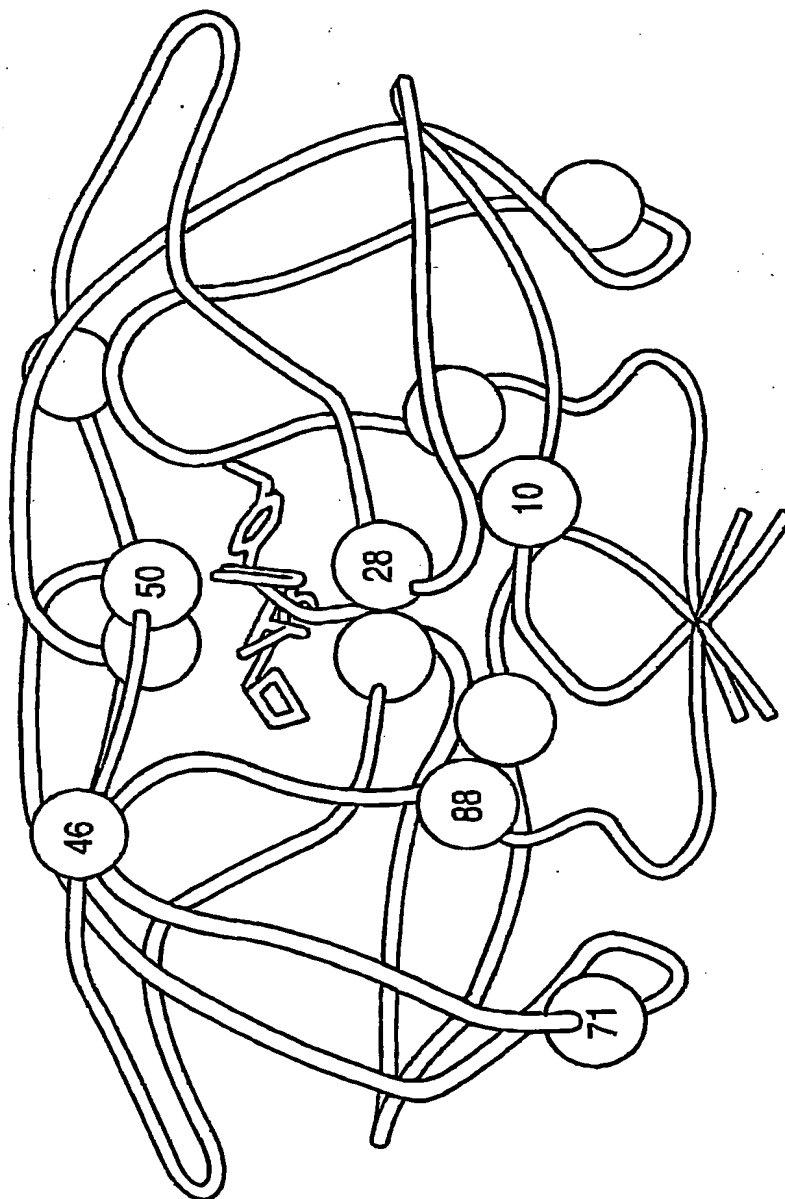
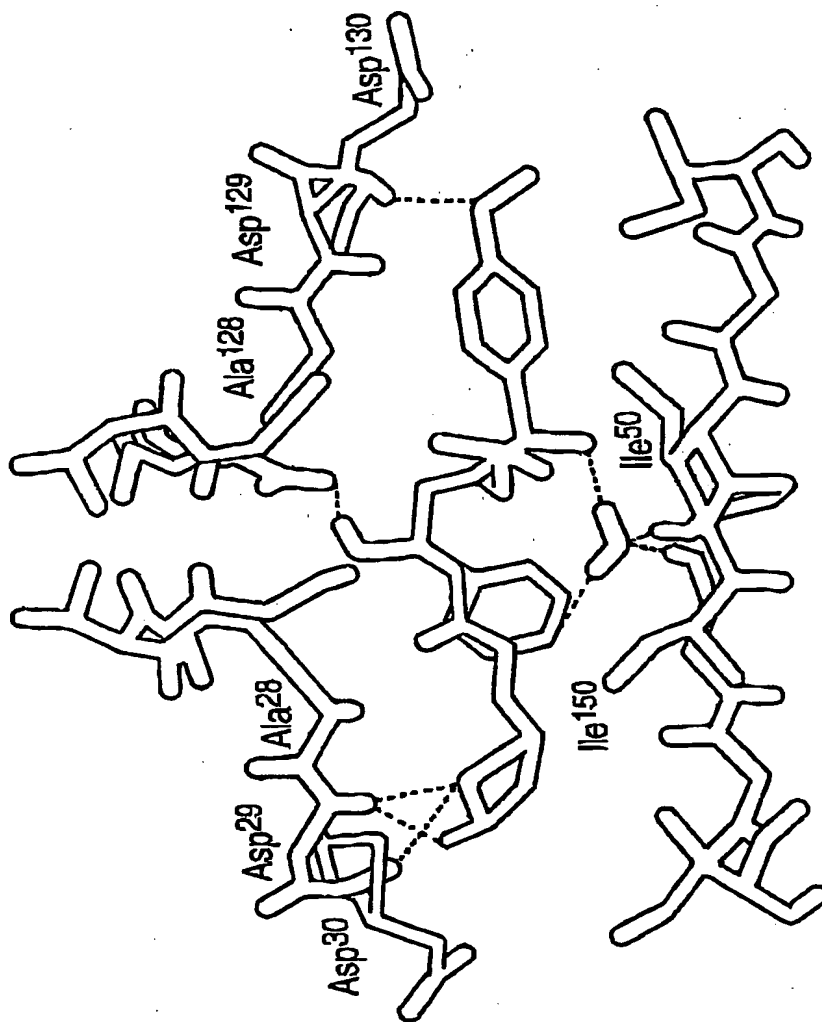
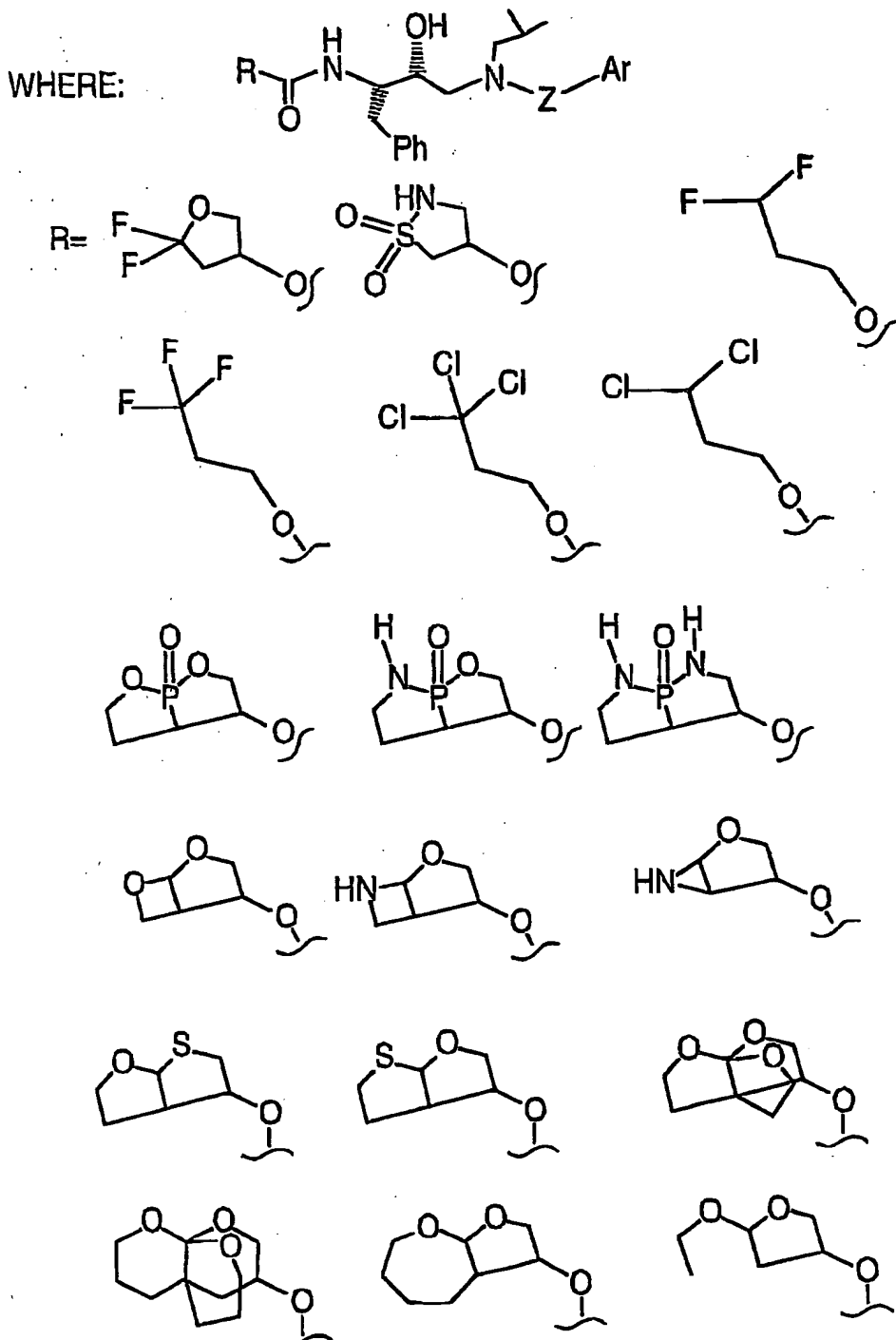


FIG. 5



8/13

10/500888

**FIG. 6**

WHERE Z IS, FOR EXAMPLE,  $-\text{S}(\text{O})_2-$ ,  $-\text{C}(\text{O})-$ ,  $-\text{C}(\text{O})-\text{NH}-$ , OR  $-\text{C}(\text{O})-\text{O}-$ , AND  
 WHERE Ar IS, FOR EXAMPLE, 4-METHOXYPHENYL, 4-AMINOPHENYL,  
 PHENYL, 4-METHYLAMINOPHENYL, OR 4-PYRIDYL



**FIG. 7A**<sup>9/13</sup>

10/500888

Table 8

Atom	x [Å]	y [Å]	z [Å]	$\sigma$ [Å]	Description
Substructure of the protein					
O301	-7.9	13.6	27.4	0.5	Oxygen atom of water molecule coordinated to main chain amide nitrogen atoms of amino acid Gly 49 and Gly149
O27	-13.8	17.7	30.4	0.5	Main Chain carbonyl oxygen atom of amino acid Gly 27
N29	-13.4	18.2	34.5	0.5	Main chain amide nitrogen atom of amino acid Asp 29
N30	-11.9	18.6	36.7	0.5	Main chain amide nitrogen atom of amino acid Asp 30
OD1 25	-11.3	21.2	28.7	0.5	Carboxylate oxygen atom of aminoacid Asp 25
OD2 25	-9.4	20.4	29.3	0.5	Carboxylate oxygen atom of aminoacid Asp 25
OD1 125	-12.7	20.3	26.4	0.5	Carboxylate oxygen atom of aminoacid Asp 125
OD2 125	-12.7	20.3	26.4	0.5	Carboxylate oxygen atom of aminoacid Asp 125
N129	-8.9	20.5	20.7	0.5	Main chain amide nitrogen atom of amino acid Asp 129
N130	-10.1	19.5	18.6	0.5	Main chain amide nitrogen atom of amino acid Asp 130
Substructure of the inhibitor					
HD:A	-8.8	17.5	25.7	0.5	Interacting with main chain carbonyl oxygen

10/13

10/500888

**FIG. 7B**

					atom of amino acid Gly 27
HA:A	-8.5	15.3	25.1	0.5	Interacting with Oxygen atom of water molecule coordinated to main chain amide nitrogen atoms of amino acid Gly 49 and Gly149
HD/A:B	-10.4	19.1	27.4	0.5	Interacting with carboxylate oxygen atoms of aminoacids Asp 25 and Asp 125
HA:A'	-8.9	14.0	29.8	0.5	Interacting with Oxygen atom of water molecule coordinated to main chain amide nitrogen atoms of amino acid Gly 49 and Gly149
HA1:X	-8.6	17.3	20.7	0.5	Main chain amide nitrogen atom of amino acid Asp 30
HA2:X	-6.9	18.7	21.4	0.5	Interacting with main chain amide nitrogen atom of amino acid Asp 29
HA:X'	-10.7	15.8	35.8	0.5	Interacting with main chain amide nitrogen atom of amino acid Asp 130

10/500888

**TABLE 1**  
SENSITIVITIES OF HIV-1<sub>LAI</sub>, HIV-1<sub>Pa-L</sub>, AND HIV-2<sub>CHO</sub> TO VARIOUS RTIs AND PIs<sup>a</sup>

VIRUS	CELLS	RTIs		PIs						
		AZT	ddI	3TC	RTV	IDV	SDV	HFV	APV	UTC-94003
HIV-1 <sub>LAI</sub>	PBMC	0.003 ± 0.0002	0.62 ± 0.02	0.021 ± 0.018	0.04 ± 0.008	0.015 ± 0.004	0.011 ± 0.005	0.009 ± 0.0003	0.017 ± 0.003	0.0003 ± 0.00009
HIV-1 <sub>Pa-L</sub>	PBMC	0.011 ± 0.007	1.5 ± 1.1	0.054 ± 0.046	0.038 ± 0.02	0.017 ± 0.011	0.014 ± 0.01	0.003 ± 0.002	0.023 ± 0.009	0.0003 ± 0.00004
HIV-1 <sub>LAI</sub>	HT-2	0.024 ± 0.003	3.4 ± 0.2	0.55 ± 0.01	0.041 ± 0.005	0.019 ± 0.009	0.023 ± 0.002	0.005 ± 0.002	0.041 ± 0.01	0.0003 ± 0.0001
HIV-2 <sub>CHO</sub>	HT-2	0.003 ± 0.001	2.8 ± 0.6	0.4 ± 0.25	0.35 ± 0.025	0.01 ± 0.004	0.004 ± 0.0005	0.02 ± 0.01	0.53 ± 0.03	0.0005 ± 0.00007

11/13

<sup>a</sup> DATA SHOWN REPRESENT MEAN VALUES (WITH STANDARD DEVIATIONS) DERIVED FROM THE RESULTS OF THREE INDEPENDENT EXPERIMENTS CONDUCTED IN DUPLICATE OR TRIPPLICATE. FOR PBMC, THE IC<sub>50</sub> WERE DETERMINED BY EMPLOYING PHA-PBMC EXPOSED TO EACH HIV-1 PREPARATION (50 TCID<sub>50</sub> PER 10<sup>5</sup> PBMC) IN THE PRESENCE OF EACH ANTI-HIV-1 AGENT AND USING THE INHIBITION OF p24<sup>ant</sup> PROTEIN PRODUCTION AS AN ENDPOINT ON DAY 7 OF CULTURE. HT-2 CELLS (2 X 10<sup>5</sup>) WERE EXPOSED TO 100 TCID<sub>50</sub> OF HIV-1<sub>LAI</sub> OR HIV-2<sub>CHO</sub> AND CULTURED IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF RTIs OR PIs, AND THE IC<sub>50</sub> WERE DETERMINED USING THE HT ASSAY ON DAY 7 OF CULTURE. ABBREVIATIONS: AZT, ZIDOVUDINE; ddI, DIDANOSINE; 3TC, LAMIVUDINE; RTV, RITONAVIR; IDV, INDINAVIR; SDV, SAQUINAVIR; HFV, NEFINAVIR; APV, AMPRENNAVIR.

10/500888

TABLE 2

SENSITIVITIES OF HIV-1 ISOLATED FROM HEAVILY DRUG-EXPERIENCED INDIVIDUALS TO PIs

VIRUS TYPE	AMINO ACID SUBSTITUTIONS IN PR-ENCODING REGION <sup>a</sup>	IC <sub>50</sub> <sup>b</sup> $\mu$ M (FOLD CHANGE)				
		RTV	IDV	SDV	NFV	APV
WILD						UIC-94003
TYPE L63P						
1	L101, K14R, L331, K361, M461, F53L, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L	0.044(1)	0.013(1)	0.010(1)	0.023(1)	0.025(1)
2	L101, K14R, L331, K361, M461, F53L, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L	>1(>23)	>1(>77)	0.27(27)	>1(>43)	0.27(11)
3	L101, K14R, L331, K361, M461, F53L, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L	>1(>23)	0.49(38)	0.037(4)	0.33(14)	0.28(11)
4	L101, K14R, L331, K361, M461, F53L, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L	>1(>23)	0.49(38)	0.036(4)	>1(>43)	0.26(10)
5	L101, K14R, L331, K361, M461, F53L, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L	>1(>23)	0.21(16)	0.033(3)	0.09(4)	0.31(12)
6	L101, K14R, L331, K361, M461, F53L, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L	>1(>23)	>1(>77)	0.31(31)	0.41(18)	0.67(27)
7	L101, K14R, L331, K361, M461, F53L, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L	>1(>23)	0.30(23)	0.19(19)	>1(>43)	0.16(6)
8	L101, K14R, L331, K361, M461, F53L, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L	>1(>23)	>1(>77)	0.12(12)	>1(>43)	0.49(20)
		>1(>23)	0.55(42)	0.042(4)	>1(>43)	0.15(6)

<sup>a</sup> THE AMINO ACID SEQUENCE OF EACH VIRAL ISOLATE WAS DEDUCED FROM THE NUCLEOTIDE SEQUENCE AND COMPARED TO THE CONSENSUS<sup>b</sup> SEQUENCE CITED FROM THE LOS ALAMOS DATA BASE. A CLINICAL ISOLATE, HIV-1<sub>BRISBANE</sub> (31), SERVED AS A SOURCE OF WILD-TYPE HIV-1.<sup>c</sup> THE IC<sub>50</sub> WERE DETERMINED BY EMPLOYING PHA-PHIC EXPOSED TO HIV-1 STRAINS (50 TCID<sub>50</sub> PER 10<sup>5</sup> PHIC) IN THE PRESENCE OF EACH ANTI-HIV-1 AGENT AND USING THE INHIBITION OF p24<sup>gag</sup> PROTEIN PRODUCTION AS AN ENDPOINT. ALL VALUES WERE DETERMINED IN TRIPLICATE, AND THOSE SHOWN ARE REPRESENTATIVE OF TWO OR THREE SEPARATE EXPERIMENTS. NUMBERS IN PARENTHESES REPRESENT FOLD CHANGES OF IC<sub>50</sub>S AGAINST EACH ISOLATE COMPARED TO IC<sub>50</sub>S AGAINST HIV-1<sub>WT</sub>.SEE TABLE 1, FOOTNOTE <sup>d</sup>, FOR ABBREVIATIONS.

13/13

10/500888

TABLE 3

AMINO ACID SUBSTITUTIONS IN PR AND SENSITIVITIES OF DRUG-RESISTANT HIV-1 STRAINS TO PIs<sup>a</sup>

VIRUS	AMINO ACID SUBSTITUTIONS	IC <sub>50</sub> , $\mu$ M (FOLD CHANGE)					
		RTV	IOV	APV	SOV	NFV	UIC-94003
HIV-1 <sub>HLA-3</sub>		0.038(1)	0.011(1)	0.042(1)	0.019(1)	0.023(1)	0.0003(1)
HIV-1 <sub>UIC-P62</sub>	L10F, A28S, M46I, I50V, A71V, N88D	0.055(1)	0.08 (7)	0.83(20)	0.01(1)	0.11(5)	0.021(70)
HIV-1 <sub>APV-P30</sub>	L10F, V32I, M46I, I54N, A71V, I84V	>1.0(>26)	0.32(30)	>1.0(>25)	0.035(2)	>1.0(43)	0.029(100)

<sup>a</sup> HT-2 CELLS (2 X 10<sup>3</sup>) WERE EXPOSED TO HIV-1<sub>HLA-3</sub>, HIV-1<sub>UIC-P62</sub>, OR HIV-1<sub>APV-P30</sub> (ALL 100 TCID<sub>50</sub>S) AND CULTURED IN THE PRESENCE OF VARIOUS DRUG CONCENTRATIONS. THE IC<sub>50</sub>S WERE DETERMINED ON DAY 7 OF CULTURE IN THE HT ASSAY. ALL VALUES WERE DETERMINED IN DUPLICATE, AND THOSE SHOWN ARE REPRESENTATIVE OF TWO OR THREE INDEPENDENT EXPERIMENTS. THE NUMBERS IN PARENTHESES REPRESENT FOLD CHANGES COMPARED TO HIV-1<sub>HLA-3</sub> (WILD TYPE). SEE TABLE 1, FOOTNOTE <sup>a</sup>, FOR ABBREVIATIONS.